

Insights into genomic DNA sampling by prokaryotic Argonaute proteins

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Prokaryotic Argonaute proteins (pAgos) are endonucleases that bind small DNA or RNA guides and mediate cleavage of complementary targets. They are encoded in a variety of bacterial and archaeal genomes and supposedly participate in cell defence against foreign DNA. Previous biochemical and structural studies have elucidated the mechanistic aspects of guide binding, target search and cleavage by pAgos. pAgos have been shown to interfere with plasmid uptake *in vivo* and to autonomously produce guides from double-stranded DNA substrates *in vitro*. However, the principles underlying self/non-self discrimination remain unknown. Here we characterize *in vivo* guide biogenesis by pAgos from mesophilic bacteria *Limnithrix rosea* (LrAgo) and *Clostridium butyricum* (CbAgo). LrAgo and CbAgo are DNA-guided DNA endonucleases that co-purify with small DNAs upon heterologous expression in *E. coli*. Such guide production depends on their catalytic activity and is abolished when pAgos are rendered inactive. Small DNAs originate from both the expression plasmid and the bacterial chromosome and are enriched for plasmid-derived sequences. Well-defined guide acquisition hotspots are observed within the host chromosome that likely correspond to the preferable sites of DNA processing by pAgos. The hotspots may presumably arise at sites of frequent DNA damage and repair and do not correlate with transcription levels at corresponding regions. Our observations suggest that pAgos may sample genomic DNA in a way similar to the CRISPR adaptation apparatus. As such the DNA repair machinery may orchestrate the action of prokaryotic defence systems by facilitating non-self targeting and guide acquisition. This work was supported by the grant of the Ministry of Science and Higher Education of the Russian Federation 14.W03.31.0007.